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Estimation of Mineral and Trace Element Profile in Bubaline Milk Affected with Subclinical Mastitis

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Abstract The milk samples from buffaloes of *Murrah* breed at mid lactation stage, reared at an organised dairy farm, were screened for subclinical mastitis based on bacteriological examination and somatic cell count following International Dairy Federation criteria. Milk samples from subclinical mastitis infected and healthy buffaloes were analysed to evaluate physicochemical alterations in terms of protein, fat, pH, electrical conductivity, chloride, minerals (sodium, potassium and calcium) and trace elements (iron, zinc, copper and selenium). In the present study, protein, fat, zinc, iron, calcium and selenium content was significantly lower ($P < 0.001$), while pH and electrical conductivity were significantly higher in mastitic milk as compared to normal milk. Concentration of electrolytes mainly sodium and chloride significantly increased with higher somatic cell count in mastitic milk and to maintain osmolality; potassium levels decreased proportionately. Correlation matrix revealed significantly positive interdependences of somatic cell count with pH, electrical conductivity, sodium and chloride. However, protein, fat, calcium and potassium were correlated negatively with elevated somatic cell count in mastitic milk. It is concluded that udder infections resulting in elevated somatic cells may alter the mineral and trace element profile of milk, and magnitude of changes may have diagnostic and prognostic value.

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Introduction

Milk is the most diversified of natural foodstuffs and is an essential, basic dietary component containing all macro (proteins, lipids and carbohydrates) and micronutrients (elements, vitamins and enzymes) required for the growth and maintenance of humans and animals [1]. India with a bubaline population of 108.7 million heads comprising 21.23 % share in total livestock of India is the first largest milk producer in the world [2]. Many intrinsic (udder health, lactation stage, age, parity and animal species) and extrinsic factors (season, nutritional status, nature of soil and locality of the farm) may regulate the microbiological and compositional quality of milk [3]. Mastitis, a multi-etiological disease of the udder parenchyma is the most prevalent production disease of buffaloes with varying degrees of clinical intensity and variations in duration. Mastitis affects the dairy industry by incurring heavy financial losses associated with reduction in milk yield, alterations in chemical composition of milk, increased treatment costs, discarded milk and loss of genetic potential of dairy buffaloes [4].

Mastitis occurs in two forms viz. clinical and subclinical. Clinical mastitis, in which changes in udder parenchyma and milk secretions are visible, and subclinical mastitis, in which no change in the milk is apparent, may both affect udder health. Subclinical mastitis is 15–40 times more prevalent than its clinical counterpart, and subclinically affected buffaloes remain a continuing source of infection for herd mates [5]. Mastitis causes a break in the blood-milk-barrier and associated with impaired synthesis and secretary activity of udder epithelial cells, which alters the level of minerals and trace elements in milk [6]. Dairy animal's immune functions are governed by irreplaceable roles of different minerals and trace elements [7]. The magnitude of changes in mineral profiles of milk varies with the severity and duration of the infection [8]. Therefore, the present study was undertaken to estimate the mineral and trace element profile in bubaline milk affected with subclinical mastitis at an organised dairy farm.

Materials and Methods

Ethical Concerns

State project on mastitis is under operation in College Central Laboratory, Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar, Haryana, India. The milk samples used in the current study were directly received from dairy owner at the time of milking and no invasive procedure was used. As per University rules, approval of Institutional Animal Ethics Committee is not required for these samples.

Sample Collection

The milk samples from buffaloes of *Murrah* breed at mid lactation stage, reared at an organised dairy farm, Haryana, India, were screened for subclinical mastitis based on bacteriological examination and somatic cell count following International Dairy Federation criteria [9] . Milkers' hands were properly washed with soap and water and teat apices sanitised with 70 % alcohol and the first few streaks were discarded. The samples were collected in morning milking shift in precleaned sterilized tubes, labelled, and immediately transported to laboratory on ice. Milk was processed for bacteriological examination, somatic cell count, pH, EC, fat, chloride content and total protein on the same day and the rest of the milk was stored at −20 °C for mineral and trace element analysis.

Milk Bacteriological Examination and Somatic Cell Count

Immediately after receiving, the 0.01 mL volume of milk samples were inoculated on 5 % sheep blood agar and MacConkey's lactose agar plates. The plates were incubated aerobically at 37 °C for 24–48 h. The somatic cell count (SCC) on milk samples was performed as described by Schalm et al. [10] after staining with modified Newman-Lampert stain (Hi media, Mumbai).

Milk Chemical Analysis

pH (pH meter, Eutech), electrical conductivity (milk checker—Eisai Co. Ltd., Tokyo, Japan), fat (Gerber method), total protein [11] and chloride content were determined in undigested raw milk immediately after receiving the samples in laboratory.

Measurements of trace elements (Fe, Zn, Se and Cu) and minerals (Na, K and Ca) were performed after microwave assisted wet digestion of milk samples employing a flame atomic absorption spectrometer (FAAS) (GBC-SensAA, Australia) and flame photometer (CL378, Elico, India) respectively. Raw milk samples measured accurately (3.0 mL each) in Teflon (XP-1500 Plus) digestion vessels were digested by employing microwave digester (CEM MarsX, USA) after the addition of 7 mL HNO_3 (71 %), filtered through 0.45-μm Whatmann filter paper and final volume was made as required. Also, the blank solutions were prepared accordingly following the same procedure, except addition of the sample. Double distilled water was used for cleaning glassware, teflon tubes, dilution of digests and preparation of standard solutions.

The equipment (FAAS) displays a double-beam optical system, multi-element hollow cathode lamps as light source, deuterium lamp for background correction and solid-state photomultiplier tube as detector. Acetylene gas was used as the fuel and air as support. Routine conditioning and optimisation procedure of the instrument before each measurement batch were performed. Digested sample solutions were nebulised, aspirated in the flame followed by introduction of de-ionised water for at least 1 min, to rinse the sampling system in order to avoid contamination of other solutions. Detailed instrumental analytical conditions for analysis of selected trace elements are given in Table 1. All working standards used for analysis were of AAS grade (certified purity >99.9 %). Analytical quality of the results was assured by subtracting the concentration of metal in blank from the analysed value in a sample. To ensure the reliability of results, standards of respective metal were run after every ten samples analysed.

Statistical Analysis

To explore any interdependences among SCC and the studied milk variables, Pearson's bivariate correlation analysis with correlation coefficient (*r*) at two-tailed significance level (*P*) was applied to the obtained data using SPSS software package (version 16.0). In order to ascertain the magnitude of variation in compositional quality between two groups, the data were subjected to Mann-Whitney test of significance.

Results and Discussion

Milk samples having somatic cell count more than five lac and positive for microbes were deemed to have subclinical mastitis [9], while those with their somatic cell count less than five lac and bacteriologically negative were considered normal.

Table 1 Instrumental analytical conditions for analysis of selected trace elements in buffalo milk samples

Element	Mode	Flame type	Background correction	Wavelength (nm)	Slit width (nm)	Gas flow (L min^{-1})	Lamp current (mA)	Detection limits (mg L^{-1})
Zn	FAAS	Air-C_2H_2	D_2 lamp	213.9	0.5	7.5	5	0.01
Cu	FAAS	Air-C_2H_2	D_2 lamp	324.7	0.5	1.8	3	0.025
Fe	FAAS	Air-C_2H_2	D_2 lamp	248.3	0.2	2.2	7	0.05
Se	HG-AAS	Air-C_2H_2	D_2 lamp	196	1.0	–	10	0.05 (μg L^{-1})

Staphylococci and streptococci were the microbes associated with subclinical mastitis in the present study. Descriptive statistics of all data regarding studied components of normal and mastitis-affected buffalo milk samples are presented in Tables 2 and 3, respectively. The negative skewness values of protein, fat, pH and selenium in normal milk suggested that their distribution was in lower range while the rest of the milk components were positively skewed and exhibited distribution towards higher range. Zn and K were positively skewed with scores clustered to the right, also Se and Cl with high positive Kurtosis value, indicated peaked distributions. Milk EC distributions show a remarkable symmetry, proved by means of the low value of 0.03 expressed by skewness statistics (Table 2).

On the contrary with negative skewness values, most of the mastitic milk components (fat, Zn, Fe, K and Cl) exhibited distribution in lower range (Table 3). The spatial variation in various milk components in both groups was very low as arithmetic and geometric mean values were nearly equal except for Na and K in mastitic milk samples which exhibited moderate variation. Lower IQR values for Se (0.04) in both groups indicated that the data points for the element are clustered around the mean value.

SCC and Milk Composition

Inflammatory diseases of udder parenchyma results decreased milk yield and also the change in the compositional quality of bovine milk because of altered membrane permeability of udder parenchyma [12]. Mean ± SD values of studied milk components of normal and mastitic milk, correlation coefficients (*r*) and differential score (*P*) between two are encapsulated in Table 4. Mean pH values significantly ($P < 0.001$) increased from 6.54 ± 0.01 in normal to 6.87 ± 0.17 in milk samples from mastitic buffaloes with higher SCC responses. Electrical conductivity showed marked and significant ($P < 0.001$) increase in mastitic milk (3.45 ± 1.03 mS/cm) as compared to normal milk (2.38 ± 0.24 mS/cm). Similar trends for pH and EC in bovine mastitic milk were documented previously in literature [13, 14]. The probable reasons for elevated levels of pH and EC in mastitic milk might be attributed to the increased sodium content in the milk, released due to increased permeability of cell membrane because of inflammatory process [14]. Also, the changes in milk pH were thought to be linked to the relocation of citrates and bicarbonates from blood to milk, during udder inflammation in response to reduced secretory activities of the mammary cells and increased permeability of the mammary epithelium [12].

It has been argued in yester studies that the mastitis affects the composition of milk, especially proteins and protease activity got altered [15]. The present findings revealed that there is significant ($P < 0.001$) decrease in a protein level from 4.44 ± 0.10 % to 3.65 ± 0.08 % with increasing SCC in mastitic milk. But contradictory to our findings, some authors reported that with the increasing level of intra mammary infections there were no changes in total protein content in mastitic milk [16, 17]. Also, an increase in milk proteins has been documented in the literature and attributed to the influx of blood-borne proteins in milk [18].

Fat content were found to be modified in the presence of intra mammary infection and significantly ($P < 0.001$) decreased from 7.08 ± 0.14 % to 6.38 ± 0.24 % with increasing SCC responses in mastitic milk. These results of the present study are in agreement with others [8, 14, 19, 20] who reported decreased fat and protein content in mastitic milk. Fat depression in milk during mastitis may be attributed to increased rate of lipolysis [21]. Depressed milk fat content in mastitic milk as compared with normal milk was not in agreement with the findings of Bruckmaier et al. [22] who reported increased milk fat content during mastitis.

Subclinical mastitis significantly altered the Ca level in milk in the present study as Ca decreased from 566 ± 90 mg/L in normal to 475 ± 77 mg/L in mastitic milk. One possible reason attributed to lowered Ca content may be the disruption of the junctional complex of mammary epithelium by udder pathogens and thus impaired permeability to calcium transport from milk to blood [23]. Lower Ca content in mastitic milk as

Table 2 Healthy buffaloes milk compositional parameters descriptive statistics

Milk components	N	Min	Max	Mean	±SD	GM	±GSD	Median	P1	P25	P75	P99	IQR (P75 − P25)	Skewness	Kurtosis
Protein (%)	20	4.30	4.60	4.44	0.10	4.43	1.02	4.50	4.30	4.30	4.50	4.60	0.20	−0.44	−1.32
Fat (%)	20	6.75	7.25	7.08	0.14	7.07	1.02	7.13	6.75	7.00	7.13	7.25	0.13	−0.68	0.22
pH	20	6.40	6.64	6.54	0.10	6.54	1.02	6.60	6.40	6.43	6.64	6.64	0.21	−0.50	−1.69
EC (mS/cm)	20	1.90	2.90	2.38	0.24	2.37	1.11	2.40	1.90	2.25	2.55	2.90	0.30	0.03	0.25
Zn (mg/L)	20	4.62	8.96	5.96	1.44	5.82	1.25	5.43	4.62	4.88	6.45	8.96	1.58	1.19	0.07
Fe (mg/L)	20	4.67	9.54	6.99	1.37	6.86	1.21	6.53	4.67	6.00	8.02	9.54	2.02	0.56	−0.59
Se (mg/L)	20	0.11	0.27	0.22	0.04	0.22	1.22	0.24	0.11	0.21	0.25	0.27	0.04	−2.06	5.74
Na (mg/L)	20	378	549	450	52	447	1.12	445	378	407	496	549	89	0.33	−1.01
K (mg/L)	20	939	1236	1063	90	1059	1.09	1027	939	1006	1126	1236	120	0.96	−0.22
Ca (mg/L)	20	438	699	566	90	559	1.18	569	438	479	651	699	172	−0.01	−1.58
Cl (mg/L)	20	532	749	616	47	614	1.08	621	532	582	641	749	59	0.68	2.37

compared to normal milk in the present study agreed with the previous studies reported in literature [8, 14, 24, 25]. However, contradictory findings reporting increased Ca content in mastitic milk were documented by Mahran et al. [26].

Blood-borne electrolytes Na and Cl significantly increased ($P < 0.001$) from 450 ± 52 to 606 ± 152 mg/L and 616 ± 47 to 828 ± 113 mg/L in mastitic milk, respectively. However, K contents decreased from 1063 ± 90 mg/L in non mastitic milk to 856 ± 156 mg/L in mastitic milk ($P < 0.001$). In line with our results, increased Na, Cl and decreased K contents in mastitic milk were reported by some authors [8, 14, 27–30]. The possible reasons for altered mineral contents may be the breakdown of ductal secretory cells and secretory epithelium, in response to microbial infection of the udder [28].

The effects of SCC responses on trace elements Zn and Fe were significant ($P < 0.001$) as the concentrations (mg/L) decreased from 5.96 ± 1.44 to 4.22 ± 0.69 and 6.99 ± 1.37 to 4.97 ± 0.76, respectively. Lower concentrations of Zn and Fe with higher SCC responses in infected quarters reported in the present study were in agreement with the results of earlier studies [8, 29, 30]. However, the reports of Guha et al. [31] and Hussain et al. [14] are contradictory to our results, where they recorded an increased iron and zinc content in mastitic buffalo milk. In an another study, trace element profile of SCM cattle milk revealed significantly increased iron, zinc and cobalt levels [32]. Also, elevated levels of milk Zn and Fe in CMT positive cattle as compared to controls were reported from Turkey [33]. Copper content in both groups was found BDL (0.05 mg/L). However, decreased Cu contents in mastitic milk were reported by some authors in yester studies [8, 14, 28]. Selenium has been recognized as an essential trace element being a part of antioxidant system of the animal body. Se levels were significantly ($P < 0.001$) lower in mastitic milk and decreased from 0.22 ± 0.04 mg/L in normal to 0.14 ± 0.06 mg/L in mastitic milk. Udder inflammation leads to oxidative stress due to production of free radicals

Table 3 Mastitic buffaloes milk compositional parameters descriptive statistics

Milk components	N	Min	Max	Mean	±SD	GM	±GSD	Median	P1	P25	P75	P99	IQR (P75 − P25)	Skewness	Kurtosis
Protein (%)	20	3.5	3.8	3.65	0.08	3.64	1.02	3.60	3.50	3.60	3.70	3.80	0.10	0.59	0.15
Fat (%)	20	6.0	6.75	6.38	0.24	6.38	1.04	6.44	6.00	6.13	6.56	6.75	0.44	−0.43	−1.07
pH	20	6.65	7.14	6.87	0.17	6.87	1.02	6.79	6.65	6.79	7.09	7.14	0.30	0.71	−1.17
EC (mS/cm)	20	2.10	6.40	3.45	1.03	3.32	1.31	3.35	2.10	2.85	3.75	6.40	0.90	1.56	3.13
Zn (mg/L)	20	2.59	4.83	4.22	0.69	4.16	1.20	4.53	2.59	3.80	4.68	4.83	0.88	−1.37	0.61
Fe (mg/L)	20	3.84	6.06	4.97	0.76	4.91	1.17	4.95	3.84	4.33	5.67	6.06	1.34	−0.06	−1.55
Se (mg/L)	20	0.09	0.27	0.14	0.06	0.13	1.39	0.12	0.09	0.11	0.14	0.27	0.04	1.78	1.92
Na (mg/L)	20	438	1052	606	152	591	1.24	590	438	515	622	1052	107	1.96	4.18
K (mg/L)	20	369	1050	856	156	837	1.26	901	369	800	943	1050	143	−1.81	4.01
Ca (mg/L)	20	395	738	475	77	470	1.16	465	395	417	510	738	94	2.15	6.52
Cl (mg/L)	20	638	1005	828	113	820	1.15	840	638	731	929	1005	198	−0.11	−1.29

Table 4 Various physicochemical components (mean ± SD), their correlation coefficients with milk SCC and differential score (*P*) of mastitic and healthy buffalo milk

Milk components	Healthy	Mastitic	Correlation coefficients (*r*)
Protein (%)	4.44 ± 0.10^{a}	3.65 ± 0.08^{b}	−0.977**
Fat (%)	7.08 ± 0.14^{a}	6.38 ± 0.24^{b}	−0.878**
pH	6.54 ± 0.01^{a}	6.87 ± 0.17^{b}	0.769**
EC (mS/cm)	2.38 ± 0.24^{a}	3.45 ± 1.03^{b}	0.589**
Zn (mg/L)	5.96 ± 1.44^{a}	4.22 ± 0.69^{b}	−0.621**
Fe (mg/L)	6.99 ± 1.37^{a}	4.97 ± 0.76^{b}	−0.682**
Na (mg/L)	450 ± 52^{a}	606 ± 152^{b}	0.577**
K (mg/L)	1063 ± 90^{a}	856 ± 156^{b}	−0.640**
Ca (mg/L)	566 ± 90^{a}	475 ± 77^{b}	−0.485**
Cl (mg/L)	616 ± 47^{a}	828 ± 113^{b}	0.782**
Se (mg/L)	0.22 ± 0.04^{a}	0.14 ± 0.06^{b}	−0.686**

Means in the same row with different superscripts are significantly significant ($P < 0.001$)

**Correlation is significant at the 0.01 level (two-tailed)a

and reactive oxygen species. Collectively, decreased trace elements in SCM result in diminished antioxidant capacity of body leading to damage of udder parenchyma [34].

Correlation between SCC and Various Milk Components

In the present study, microbiological and biochemical components of milk were significantly altered by higher SCC responses; Pearson correlation technique was applied to explore any interdependencies among them and the results of correlation matrix are given in Table 4. Somatic cell count (SCC) showed significantly positive interdependences with pH (r = 0.769**, $P < 0.01$), EC (r = 0.589**, $P < 0.01$), milk electrolytes Na (r = 0.577**, $P < 0.01$) and Cl (r = 0.782**, $P < 0.01$) but showed significantly negative interaction with milk mineral Ca (r = −0.485**, $P < 0.01$), K (r = −0.640**, $P < 0.01$), protein (r = −0.977**, $P < 0.01$) and fat (r = −0.878**, $P < 0.01$) content of milk. These results of correlation matrix are comparable with Ogola et al. [35] who reported a positive correlation with Na ($r = 0.87$) and Cl ($r = 0.85$) and a negative correlation with K ($r = -0.64$) with higher SCC responses in infected quarters. The SCC and trace minerals, Fe (r = −0.682**, $P < 0.01$), Zn (r = −0.621**, $P < 0.01$) and Se (r = −0.686**, $P < 0.01$) were found correlated negatively at two-tailed level of significance. Also, Na content was found significantly and positively correlated with Cl ($r = 0.727$, $P < 0.01$) but negatively correlated with K content ($r = -0.680$, $P < 0.01$) of milk and were in accordance with the results reported by others [22, 35].

Conclusion

Present study concluded that bubaline milk affected with subclinical mastitis had significantly lower protein, fat, zinc, iron, calcium and selenium content while significantly higher pH and EC as compared to normal milk. Blood-borne electrolytes sodium and chloride were found elevated in mastitic milk and to maintain osmolality; K levels decrease proportionately. In conclusion, the magnitude of changes in mineral and trace element profiles in milk of subclinically infected buffaloes in comparison to healthy buffaloes suggested diagnostic and prognostic value.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

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